DOCKET NO.: CACO-0067 (P21303US) PATENT APPLICATION

INT'L. SERIAL NO.: PCT/GB00/00157 INT'L. APPL. FILED: JANUARY 21, 2000

At page 13, replace the paragraph spanning lines 18 - 24 with the following replacement paragraph:

A DNA fragment containing the tac promoter was obtained from plasmid pDR540 (Pharmacia), by PCR amplification using primers 5'- ACCTGACGTCTAAGAAAC -3' (SEQ ID NO:1) and 5'-GCTCTAGATTGTTATCCGCTCAC -3' (SEQ ID NO:2). The amplified DNA fragment was cleaved with restriction endonucleases *Eco*RI and *Xba*I, and the major fragment (369 bp) was cloned between the *Eco*RI and *Xba*I sites of the widely used vector plasmid, pUC19, to generate plasmid pPS1133C2. The sequence of the insert was verified by DNA sequencing (Sanger, *et al.* 1977, Proc. Natl. Acad. Sci. USA, 74: 5463-7).

At page 14, replace the paragraph spanning lines 2 - 7 with the following replacement paragraph:

The two oligonucleotides PS1133A (5'- CTAGGGCCTGCGAGGCCTTAATTAA-GGCCTCCCGGGGCCT -3') (SEQ ID NO:3) and PS1133B (5'-CTAGAGGCCCGGGAGGCCTTAATTAAGGCCTCGCAGGCC -3') (SEQ ID NO:4) were annealed together to generate a short piece of DNA containing two *Sfi*I sites separated by a *Pac*I site, and with 4 nucleotide 5' extensions on either end compatible with ligation into *Xba*I sites. However, only that at the right end regenerates the *Xba*I site:

At page 14, replace the paragraph spanning lines 17 - 20 with the following replacement-paragraph:

The plasmid pET11c (Novagen) was cleaved with XbaI, and the following annealed oligonucleotides were cloned into that site:

PS1134A 5' CTAGAGGCCTGCGAGGC 3' (SEQ ID NO:5) PS1134B 3' TCCGGACGCTCCGGATC 5' (SEQ ID NO:6)

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Replace the paragraph spanning from page 14, line 25 through page 15, line 2 with the following replacement paragraph:

Plasmid pPS1134D4 was cleaved with BamHI, and the following annealed oligonucleotides were cloned into that site:

GATCCGGCCTCCCGGGCC

(SEQ ID NO:7)

PS1134D 3'

GCCGGAGGCCCGGCTAG 5' (SEQ ID NO:8)

At page 15 replace the paragraph spanning lines 14 - 18 with the following replacement paragraph:

DNA sequence derived from the nitroreductase gene (nfnB) of E. coli strain DH5α was amplified by the polymerase chain reaction from genomic DNA purified from that strain, using primers PS1138A 5'- GGGAATTCCATATGGATATCATTTCTGTCGCCTTAAAGC-3' (SEQ ID NO:9) and

PS1138B 5'- CGCGGATCCTGAGAGGAAATAGCCGGGCAGATGC -3' (SEQ ID NO:10).

At page 17 replace the paragraph spanning lines 17 - 24 with the following replacement paragraph:

The polymerase chain reaction (PCR) was used to determine whether the bacteria in each colony contained \(\lambda JG3J1 \) or \(\lambda JG16C1. \) A number of individual colonies were picked at random from the most relevant plates and transferred to a 200µl PCR tube, lysed in a microwave for 2min and 35µl of PCR reaction mix [16mM (NH₄)₂SO₄, 67mM Tris-Cl (pH 8.8 at 25°C), 0.01% Tween-20 (Bioline), 0.2mM each dNTP, 1.5mM MgCl₂, 1U Biotaq (Bioline)] plus 0.25 μM primers JG2A 5'-TGGCGGAAAGGTATGCATGC-3' (SEQ ID NO:11) and; JG2B 5'-CAGAGCATTAGCGCAAGGTG-3' (SEQ ID NO:12),

which anneal to λ sequences flanking the *Hin*dIII site, was added.